

Amendments to the Specification

Please replace the paragraph appearing on page 7, beginning on line 13 with the following:

P. While it has been documented that MMR deficiency can lead to as much as a 1000-fold increase in the endogenous DNA mutation rate of a host, there is no assurance that MMR deficiency alone will be sufficient to alter every gene within the DNA of the host bacterium to create altered biochemicals with new activity(s). Therefore, the use of chemical agents and their respective analogues such as ethidium bromide, EMS, MNNG, MNU, Tamoxifen, 8-Hydroxyguanine, as well as others listed but not limited to in publications by: Khromov-Borisov, N. N., et.al. (Mutat. Res. 430:55-74, 1999); Ohe, T., et.al. (Mutat. Res. 429:189-199, 1999); Hour, T. C. et.al. (Food Chem. Toxicol. 37:569-579, 1999); Hrelia, P., et.al. (Chem. Biol. Interact. 118:99-111, 1999); Garganta, F., et.al. (Environ. Mol. Mutagen. 33:75-85, 1999); Ukawa-Ishikawa S., et.al. (Mutat. Res. 412:99-107, 1998); www.ehs.utah.edu/ohh/mutagens the website having the URL address: www host server, ehs.utah.edu domain name, ohh directory, mutagens subdirectory, etc. can be used to further enhance the spectrum of mutations and increase the likelihood of obtaining alterations in one or more genes that can in turn generate host bacteria with a desired new output trait(s) (10,39,40). Prior art teaches that mismatch repair deficiency leads to hosts with an increased resistance to toxicity by chemicals with DNA damaging activity. This feature allows for the creation of additional genetically diverse hosts when mismatch defective bacteria are exposed to such agents, which would be otherwise impossible due to the toxic effects of such chemical mutagens [Colella, G., et.al. (Br. J. Cancer 80:338-343, 1999); Moreland, N. J., et.al. (Cancer Res. 59:2102-

2106, 1999); Humbert, O., et.al. (Carcinogenesis 20:205-214, 1999); Glaab, W. E., et.al. (Mutat. Res. 398:197-207, 1998)]. Moreover, prior art teaches that mismatch repair is responsible for repairing chemical-induced DNA adducts, so therefore blocking this process could theoretically increase the number, types, mutation rate and genomic alterations of a bacterial host [Rasmussen, L. J. et.al. (Carcinogenesis 17:2085-2088, 1996); Sledziewska-Gojska, E., et.al. (Mutat. Res. 383:31-37, 1997); and Janion, C. et.al. (Mutat. Res. 210:15-22, 1989)]. In addition to the chemicals listed above, other types of DNA mutagens include ionizing radiation and UV-irradiation, which are known to cause DNA mutagenesis in bacteria can also be used to potentially enhance this process. These agents which are extremely toxic to host cells and therefore result in a decrease in the actual pool size of altered bacterial cells are more tolerated in MMR defective hosts and in turn allow for a enriched spectrum and degree of genomic mutation (7).

Please replace the paragraph appearing on page 11, beginning on line 25 with the following:

Dominant negative alleles of a mismatch repair gene can be obtained from the cells of humans, animals, yeast, bacteria, plants or other organisms. Screening cells for defective mismatch repair activity can identify such alleles. Mismatch repair genes may be mutant or wild type. Bacterial host MMR may be mutated or not. The term bacteria used in this application include any organism from the prokaryotic kingdom. These organisms include genera such as but not limited to *Agrobacterium*, *Anaerobacter*, *Aquabacterium*, *Azorhizobium*, *Bacillus*, *Bradyrhizobium*, *Cryobacterium*, *Escherichia*, *Enterococcus*, *Heliobacterium*, *Klebsiella*, *Lactobacillus*, *Methanococcus*,

BEST AVAILABLE COPY

Methanothermobacter, Micrococcus, Mycobacterium, Oceanomonas, Pseudomonas, Rhizobium, Staphylococcus, Streptococcus, Streptomyces, Thermusaquaticus, Thermaerobacter, Thermobacillus, etc. Other procaryotes that can be used for this application are listed at ~~(www.bacterio.eiet.fr/validgenericnames)~~ the website having the URL address www host server, bacterio.cict.fr domain name, validgenericnames directory. Bacteria exposed to chemical mutagens or radiation exposure can be screened for defective mismatch repair. Genomic DNA, cDNA, or mRNA from any cell encoding a mismatch repair protein can be analyzed for variations from the wild type sequence.

D2 conc. 1

Dominant negative alleles of a mismatch repair gene can also be created artificially, for example, by producing variants of the hPMS2-134 allele or other mismatch repair genes (32). Various techniques of site-directed mutagenesis can be used. The suitability of such alleles, whether natural or artificial, for use in generating hypermutable bacteria can be evaluated by testing the mismatch repair activity (using methods described in ref 32) caused by the allele in the presence of one or more wild-type alleles, to determine if it is a dominant negative allele.

Please replace the paragraph appearing on page 33, beginning on line 22 with the following:

P3

It has been previously documented that MMR deficiency yields to increased mutation frequency and increased resistance to toxic effects of chemical mutagens (CM) and their respective analogues such as but not limited to those as: ethidium bromide, EMS, MNNG, MNU, Tamoxifen, 8-Hydroxyguanine, as well as others listed but not limited to in publications by: Khromov-Borisov, N. N., et.al. (Mutat. Res. 430:55-74,

BEST AVAILABLE COPY

1999); Ohe, T., et.al. (Mutat. Res. 429:189-199, 1999); Hour, T. C. et.al. (Food Chem. Toxicol. 37:569-579, 1999); Hrelia, P., et.al. (Chem. Biol. Interact. 118:99-111, 1999); Garganta, F., et.al. (Environ. Mol. Mutagen. 33:75-85, 1999); Ukawa-Ishikawa S., et.al. (Mutat. Res. 412:99-107, 1998); www.ehs.utah.edu/ohh/mutagens
www.ehs.utah.edu/ohh/mutagens the website having the URL address: www host server, ehs.utah.edu domain name, ohh directory, mutagens subdirectory, etc. To demonstrate the ability of CMs to increase the mutation frequency in MMR defective bacterial cells, we exposed T7-PMS134 BL21 cells to CMs.

Q3
conc d

BEST AVAILABLE COPY